



Contents lists available at SciOpen

Food Science and Human Wellness

journal homepage: <https://www.sciopen.com/journal/2097-0765>

Akkermansia muciniphila PROBIO ameliorates overweight via gut microbiota modulation: a randomized controlled trial

Jie You^{#a,b,c}, Wu Liu^{#d}, Yuxiong Huang^e, Di Zhu^e, Xuan Xu^f, Yufan Wang^e, Jianying Chen^e,
Qingling Yang^d, Tangchang Xu^g, Weijun Chen^h, Yifeng Yu^{*a}, Tingtao Chen^{*a,b,c}

^aOphthalmologic Center, The Second Affiliated Hospital, Jiangxi Medical College, Nanchang University, Nanchang, China

^bSchool of Pharmacy, Jiangxi Medical College, Nanchang University, Nanchang 330031, China.

^cJiangxi Province Key Laboratory of Bioengineering Drugs, Institute of Translational Medicine, Jiangxi Medical College, Nanchang University, Nanchang 330031, China P. R. China.

^dThe Second Affiliated Hospital of Nanchang University, No.1 Minde Road, Nanchang 330000, P.R. China.

^eQueen Mary School, Jiangxi Medical College, Nanchang University, Nanchang 330031, China.

^fSchool of Huankui Academy, Nanchang University, Nanchang 330031, China.

^gSchool of Life Science, Nanchang University, Nanchang 330031, China.

^hThe Reproductive Hospital, Jiangxi University of Traditional Chinese Medicine, Nanchang 330004, China.

ABSTRACT: As a next-generation probiotic, the therapeutic advantages of *Akkermansia muciniphila* (*A. muciniphila*) in metabolic diseases have been widely demonstrated. However, the exclusion of *A. muciniphila* from the List of Approved Food Microbial Strains in China creates regulatory barriers for conducting clinical trials, leading to a significant lack of clinical evidence. In this study, 130 overweight participants were randomly assigned to receive a placebo (PLA), viable *A. muciniphila* PROBIO (PB), or *A. muciniphila* PROBIO postbiotics (POST) intervention for 8 weeks. Our results demonstrated that, after 8 weeks of intervention, both the PB group (PB vs PLA = -0.63 vs 1.272, $P < 0.001$) and the POST group (POST vs PLA = -0.63 vs 0.43, $P < 0.001$) demonstrated significantly greater weight reduction compared to the PLA group. Additionally, the viable *A. muciniphila* PROBIO and postbiotics interventions significantly improved liver function, lipid metabolism, emotional well-being, and gut microbiota composition. Notably, the viable *A. muciniphila* intervention significantly enhanced key metabolic parameters, including total bilirubin ($p = 0.031$), triglycerides ($P < 0.001$), total cholesterol ($p = 0.023$), and LDL-C ($p = 0.044$), demonstrating an overall efficacy that exceeds its postbiotics, which contradicts current mainstream views. In conclusion, this study systematically evaluated the effects of viable *A. muciniphila* PROBIO and its postbiotics in overweight individuals, confirming their ability to improve overweight-related metabolic disorders through various mechanisms, including reducing lipid accumulation, modulating liver function, and reshaping gut microbiota. These findings provide a theoretical foundation and data support for developing products and clinical applications of *A. muciniphila* PROBIO in managing metabolic disorders such as overweight and obesity.

Keywords: Overweight, *Akkermansia muciniphila* PROBIO, Probiotics, Postbiotics, Gut microbiota.

1. Introduction

[#] These authors contributed equally to this work.

***Corresponding author**

chentingtao1984@163.com or chentingtao@ncu.edu.cn (Tingtao Chen); 171018170@qq.com (Yifeng Yu)

Received 18 April 2025

Received in revised form 20 May 2025

Accepted 11 June 2025

Overweight is a chronic metabolic disorder characterized by the excessive accumulation of body fat. The WHO data indicate that more than 1.9 billion adults globally suffer from overweight, which has emerged as a critical challenge to international public health^[1]. The prevalence of overweight continues to increase annually, with projections suggesting over 2 billion people worldwide will suffer from overweight or obese by 2035^[2]. Without timely intervention, overweight can result in serious health conditions, including hypertension, coronary heart disease, heart failure, and exacerbate mood disorders such as anxiety and depression^[3, 4]. These complications not only significantly impair patients' quality of life but also endanger their health and survival. Current obesity management primarily relies on dietary control and pharmacological interventions. Although the U.S. Food and Drug Administration (FDA) has approved anti-obesity drugs like Orlistat and Phentermine-Topiramate, their clinical application remains limited due to modest efficacy and potential adverse effects, including steatorrhea, abdominal pain, and insomnia^[5]. Therefore, the development of safer and more effective therapeutic strategies holds significant clinical importance.

Probiotics are defined as live microorganisms that confer health benefits to the host when administered in adequate amounts^[6]. Numerous studies demonstrated that probiotics comprehensively ameliorated overweight and related mood disorders through multiple mechanisms, including restoring gut microbiota balance^[7], repairing intestinal barrier^[8], enhancing energy metabolism^[9], alleviating inflammation^[10], and regulating hormone levels^[11]. Currently, conventional probiotics have been widely applied for the treatment of metabolic disorders including overweight and obesity. Clinical studies demonstrated that live bacterial preparations like *Lactobacillus fermentum* and *Lactobacillus plantarum* provide significant benefits in modulating gut microbiota, reducing body fat, regulating blood lipids, and improving insulin resistance in overweight patients^[12-14]. These studies suggest probiotic live preparations have promising potential for application as dietary supplements in the treatment of metabolic diseases.

Traditional probiotics have been extensively studied in metabolic regulation. However, clinical evidence supporting their direct weight-loss effects remains limited. As a next-generation probiotic, *Akkermansia muciniphila* (*A. muciniphila*) has demonstrated significant therapeutic effects on metabolic disorders, including obesity, type 2 diabetes, hypertension, as well as neurological conditions like Parkinson's disease and autism^[15]. Notably, a clinical trial demonstrated that pasteurized *A. muciniphila* not only effectively reduced body weight and fat percentage in overweight/obese patients, but also significantly enhanced insulin sensitivity, improved liver dysfunction, and alleviated chronic inflammation^[16]. In addition, an animal study demonstrated *A. muciniphila* ameliorated high-fat-diet-induced obesity by downregulating perilipin2 expression in both brown and white adipose tissues, while also reducing intestinal carbohydrate absorption^[17]. Based on the above evidences, *A. muciniphila* shows significant clinical potential for the prevention and treatment of metabolic disorders.

Although previous studies have shown that inactivated *A. muciniphila* can significantly improve metabolic parameters in obese and overweight patients, with effects superior to viable preparations^[16],

recent findings have shown that viable *A. muciniphila* also exhibits significant efficacy in overweight and obese individuals with low baseline gut colonization levels of this bacterium^[18]. Nevertheless, there remains a lack of comprehensive and systematic evidence regarding the precise therapeutic effects of viable *A. muciniphila* on overweight patients and its comparative advantages over inactivated preparations.

Therefore, a randomized controlled trial was designed, which included 130 overweight individuals and another 30 normal people control for microbial detection. The aim of this trial is to systematically evaluate the effects of viable *A. muciniphila* PROBIO and its postbiotics on alleviating overweight, while thoroughly comparing the therapeutic differences between viable *A. muciniphila* PROBIO and its postbiotic. The study utilized high-throughput sequencing technology to comprehensively analyze the regulatory effects of viable *A. muciniphila* PROBIO and its postbiotics on gut microbiota diversity and compositional structure. The study not only provides scientific evidence regarding the effects of *A. muciniphila* PROBIO on improving overweight and associated anxiety/depression symptoms, but also offers a safe and effective intervention strategy for the clinical treatment of overweight.

2. Materials and methods

2.1 Trial design and participants

A randomized, double-blind, placebo-controlled trial was conducted at Nanchang Reproductive Hospital from October 2024 to January 2025. The participants recruited for this trial were overweight. All procedures were reviewed and approved by the Medical Ethics Committee of Nanchang Reproductive Hospital (Approval No. [2024] 026) and were performed in accordance with the approved guidelines. All participants in this clinical trial provided voluntary informed consent by signing the necessary documentation. And all participants are from Nanchang Reproductive Hospital.

The inclusion criteria for overweight participants included: (1) individuals with overweight (BMI ≥ 24.0 kg/m²); (2) aged between 18 and 35 years; (3) willingness to participate in the study and provision of informed consent. The exclusion criteria for overweight participants included: (1) individuals with severe organic or chronic diseases affecting the heart, liver, kidneys, or blood systems; (2) use of medications influencing weight, blood glucose, or lipid levels in the past 3 months; (3) pregnancy; (4) prior weight-loss surgery; (5) inability to complete physical function tests or accurately respond to questionnaires; (6) non-compliance with trial protocols; (7) allergic reactions to the test product components; (8) refusal to provide informed consent; (9) use of weight-loss, anti-anxiety, or antidepressant medications or participation in weight-loss programs with a nutritionist within the last 3 months.

The inclusion criteria for healthy people included: (1) individuals with the BMI range (18.5-23.9 kg/m²); (2) Aged 18-35 years; (3) voluntarily participated in this study and provided written informed consent. The exclusion criteria for healthy people included: (1) individuals with severe organic or chronic diseases affecting the heart, liver, kidneys, or blood systems; (2) use of medications influencing weight, blood glucose, or lipid levels in the past 3 months; (3) pregnancy; (4) prior weight-loss surgery; (5) inability to complete physical function tests or accurately respond to questionnaires; (6) non-compliance with trial protocols; (7) allergic

reactions to the test product components; (8) refusal to provide informed consent; (9) use of weight-loss, anti-anxiety, or antidepressant medications or participation in weight-loss programs with a nutritionist within the last 3 months.

This randomized controlled trial was designed and reported in accordance with the CONSORT 2010 guidelines. The completed CONSORT checklist and flow diagram are provided in Supplementary Materials Table S2. All study procedures adhered to the principles of the Declaration of Helsinki. Furthermore, this study was registered in the Chinese Clinical Trials Registration Center (ClinicalTrials.gov) under the unique identifier ChiCTR2500095821.

2.2 Randomization and masking

The staff not involved in the clinical trial used an online randomization tool to allocate all participants to the placebo (PLA) group, viable bacteria (PB) group, and postbiotic (POST) group in a 3:5:5 ratio. The assigned staff labelled the placebo, viable bacteria, and postbiotics with unique, consecutive numerical codes following a randomized sequence. The packaging and odour of the three types of products were identical to ensure complete concealment of allocation. Throughout the experiment, both researchers and participants were blinded to the group assignments. The viable *A. muciniphila* PROBIO, *A. muciniphila* PROBIO postbiotics, and placebo were all provided by Thankcome Research Center for Human Microbiota and Nutritional Health (Suzhou).

2.3 Intervention procedure

A. muciniphila PROBIO (CGMCC No.20955) is a probiotic strain screened and isolated from healthy individuals. Each capsule of the viable *A. muciniphila* PROBIO product contains 1×10^{10} bacteria of viable *A. muciniphila* PROBIO, with maltodextrin used as an excipient. Each capsule of the *A. muciniphila* PROBIO postbiotics product contains 1×10^{10} bacteria of pasteurized *A. muciniphila* PROBIO. The *A. muciniphila* PROBIO formulations were prepared through distinct processes for postbiotic and viable forms. For the postbiotic preparation, following high-density fermentation, *A. muciniphila* PROBIO cells were harvested and pasteurized (80-90°C, 30 min) to ensure complete inactivation while preserving bioactive components; the inactivated biomass was standardized with maltodextrin and encapsulated in gelatin-hydroxypropyl methylcellulose dual-layer capsules, yielding 1×10^{10} bacteria-equivalent per capsule. For viable *A. muciniphila* PROBIO, cultures were grown anaerobically in mucin-based medium to late-log phase, harvested via centrifugation, washed twice with PBS, resuspended, blended with maltodextrin, and encapsulated in enteric-coated hydroxypropyl methylcellulose capsules at 1×10^{10} bacteria/capsule, with viability confirmed by plate counting. Both processes maintained component integrity while ensuring precise dosage control. The placebo capsules contain only an equivalent amount of maltodextrin. All three types of products are encapsulated in capsules made of gelatin and hydroxypropyl methylcellulose, with all capsules being light yellow in color and having the same taste. This study enrolled 130 overweight participants as subjects, with 30 randomly assigned to the PLA group receiving placebo, 50 to the PB group receiving viable probiotics, and 50 to the POST group receiving postbiotic treatment. Ultimately 124

participants completed the study (PLA: n=30; PB: n=47; POST: n=47). In addition, this study included 30 healthy individuals as a control group (HP) for gut microbiota analysis. During the 8-week intervention, each participant took one capsule orally per day. All participants were required to maintain their normal dietary habits and physical activity levels during the study to avoid any weight fluctuations caused by changes in diet or physical activity. Various parameters were assessed before and after the intervention in all groups.

2.4 Primary Outcomes

The primary outcome was the change in anthropometric measurements, including body weight, BMI, body fat percentage, waist circumference (Waist), hip circumference (Hip) and waist-to-hip ratio (WHR) at 8 weeks compared to baseline. During each follow-up, professional staff measured and documented various anthropometric parameters. Participants' body weight was measured using a calibrated portable digital scale (SECA, Hamburg, Germany). Professional staff measured participants' height using a portable stadiometer (SECA, Hamburg, Germany) while participants stood upright, with measurements precise to 0.1 cm. BMI (kg/m^2) was determined using the formula: $\text{BMI} = \text{weight}/\text{height}^2$. Waist circumference and Hip circumference were measured directly by professional staff using a non-elastic tape measure following normal exhalation by participants. The waist-to-hip ratio was computed as: $\text{WHR} = \text{Waist circumference} / \text{Hip circumference}$.

2.5 Secondary Outcomes

Secondary outcomes included body fat percentage, fasting blood glucose, lipid profile, liver function tests, blood glucagon-like peptide-1 (GLP-1), serum Leptin (LEP), serum Peptide YY (PYY), serum inflammatory factors (IL-6, IL-1 β), 5-hydroxytryptamine (5-HT), and dopamine (DA). Body fat was measured using bioelectrical impedance analysis (BIA) for body composition assessment. Fasting blood glucose was measured using an automated analyzer (Roche/Hitachi Modular P800). The lipid profile, including triglycerides (TG), total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), and high-density lipoprotein cholesterol (HDL-C), was assessed using a Beckman Coulter D600 analyzer (Brea, California, USA). Liver function tests, including alanine aminotransferase (ALT), aspartate aminotransferase (AST), total bilirubin, direct bilirubin, and indirect bilirubin, were performed using an ICG liver function reserve analyzer. Serum levels of GLP-1, IL-6, IL-1 β , 5-HT and DA were measured using ELISA kits (Supplementary Material Table S1). The anxiety level, depression level, and sleep status of overweight patients were assessed by using the 14-item Hamilton Anxiety Rating Scale (HAMA-14), the 17-item Hamilton Depression Rating Scale (HDRS-17), and the Pittsburgh Sleep Quality Index (PSQI), respectively.

2.6 High throughput sequencing

After 8 weeks of intervention, fecal samples were collected, stored in sterile containers, and immediately preserved in a -80 °C freezer. Genomic DNA was extracted from fecal samples using a genomic DNA kit (M5636-02, Omega, USA), and DNA concentration was measured using a NanoDrop spectrophotometer

(ThermoFisher, Canada). This study utilized 16S rRNA gene sequencing technology to analyze the gut microbiota. The highly variable V4 region of the 16S rDNA was amplified using polymerase chain reaction (PCR). To ensure data quality, sequences underwent quality control procedures using the DADA 2 method, followed by the generation of denoised amplicon sequence variants (ASVs). These ASVs were rigorously analyzed using the QIIME 2 software package. Finally, taxonomic classification of these ASVs was completed by referencing the GreenGenes database. Alpha diversity indices of the gut microbial communities were calculated, and intergroup comparisons were performed using the Kruskal-Wallis test. Beta diversity of the microbial communities was assessed using principal coordinate analysis (PCoA) based on Unweighted UniFrac and Jaccard distances. Differential relative abundances of bacterial taxa among groups were determined using linear discriminant analysis effect size (LEfSe). Microbial abundances across the three groups were compared using the non-parametric Kruskal-Wallis test. The raw high-throughput sequencing data from this clinical trial have been uploaded to the NCBI database (PRJNA1251145).

2.7 Statistical analyzes

The target number of participants required was calculated based on previous studies using other strains of *A. muciniphila*. According to the study by Clara Depommier et al., treatment with viable *A. muciniphila* for 12 weeks resulted in a weight reduction of (1.73 ± 0.64) kg from baseline^[16]. Based on these data, assuming a statistical power of 0.80, a two-sided significance level of 0.05, and a dropout rate of 20%, we determined the minimum required sample sizes to be 26 participants for the placebo group and 44 participants each for the viable *A. muciniphila* and inactivated *A. muciniphila* intervention groups, yielding an allocation ratio of 3:5:5. This calculation ensures adequate power to detect clinically meaningful differences between study groups while accounting for potential participant attrition.

3. Results

3.1 Baseline characteristics of participants

This study enrolled 130 overweight patients who were randomly assigned to PLA, PB, or POST groups. During the intervention, 3 participants from the probiotic group and 3 from the postbiotic group withdrew due to scheduling conflicts. Ultimately, 124 participants were included in the analysis: 30 in placebo, 47 in probiotic, and 47 in postbiotic groups (Fig. 1). The mean ages were 20.27, 20.00, and 20.13 years in the placebo, probiotic, and postbiotic groups, respectively. Gender distribution showed female percentages of 43.3%, 53.2%, and 48.9% in the placebo, probiotic, and postbiotic groups. There are no significant differences existing in mean BMI values: 26.69 ± 1.63 , 27.50 ± 3.06 , and 27.43 ± 2.24 between the three groups. The three groups demonstrated similar baseline characteristics with high homogeneity (Table 1).

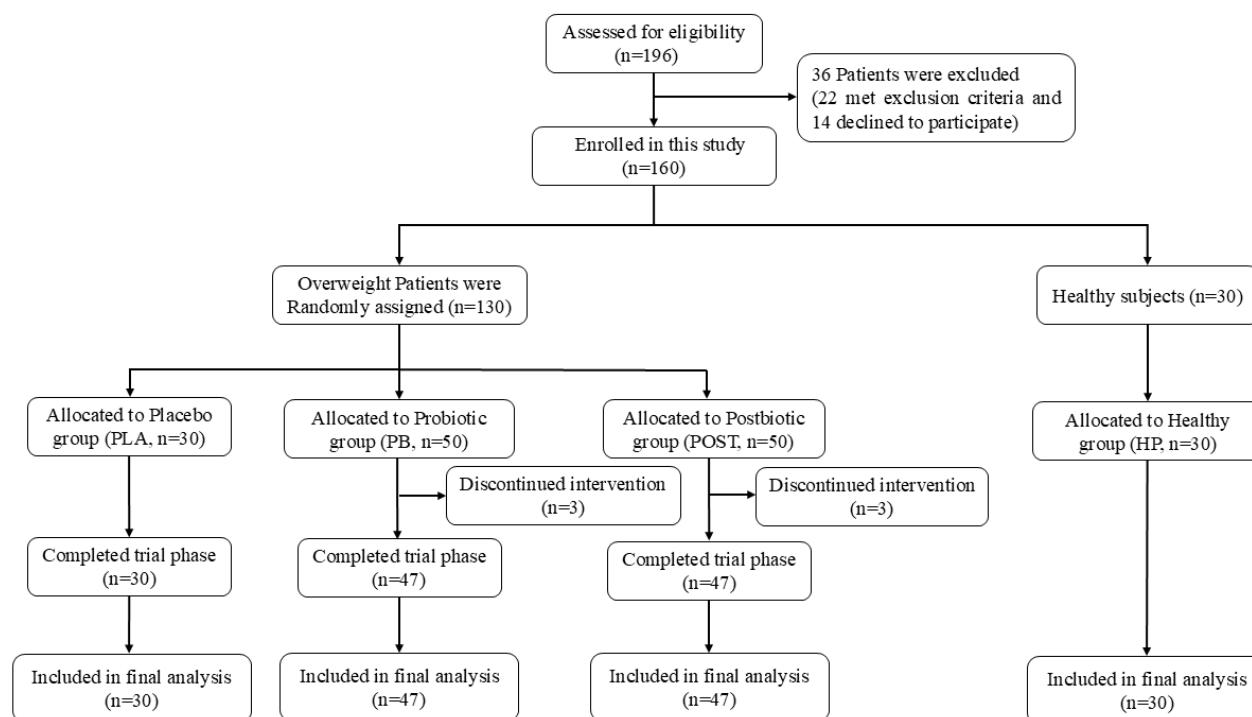


Fig. 1 Flow chart of patient recruitment and randomization.

Table 1 Baseline characteristics of patients

Characteristics	PLA (n = 30)	PB (n = 47)	POST (n = 47)	<i>P</i> _{total}
Age	20.27±1.34	20.00 ± 1.08	20.13 ± 1.30	0.65
Female/Male (n)	13/17	25/22	23/24	
Height (m)	1.71 ± 0.07	1.70 ± 0.08	1.71 ± 0.08	0.97
Body Weight(kg)	77.96 ± 9.30	80.04 ± 10.9	80.35 ± 10.18	0.58
Fat mass (kg)	23.5 (2.77)	23.4 (8.00)	22.7 (5.90)	0.80
BMI (kg/m ²)	26.69 ± 1.63	27.50 ± 3.06	27.43 ± 2.24	0.34
Waist (cm)	92.17 ± 6.87	93.33± 9.29	93.46 ± 9.30	0.80
Hip (cm)	102.89 ± 4.41	101.78 ± 6.01	103.3 ± 6.05	0.42
Waist-hip ratio	0.89 (0.05)	0.89 (0.12)	0.90 (0.07)	0.62
HAMA-14a	14.33 ± 5.65	13.43 ± 5.26	14.13 ± 5.32	0.73
HDRS-17b	12.87 ± 6.40	11.81 ± 6.44	12.72 ± 7.23	0.74
PSQI	12.87 ± 4.55	14.02 ± 4.22	13.06 ± 4.48	0.44
AST (U/L)	19.43 (9.30)	23.42 (13.64)	21.37 (11.05)	0.02*
ALT (U/L)	26.17 ± 10.01	29.3 ± 11.8	32.48 ± 12.23	0.07
Total Bilirubin (mg/dL)	0.57 ± 0.18	0.68 ± 0.22	0.62 ± 0.19	0.06
Direct Bilirubin (mg/dL)	0.26 ± 0.09	0.29 ± 0.10	0.27 ± 0.07	0.15
Indirect Bilirubin (mg/dL)	0.32 ± 0.22	0.39 ± 0.18	0.35 ± 0.20	0.24
Fasting Blood Glucose (mg/dL)	100.41 ± 9.05	91.62 ± 5.52	91.46 ± 5.73	0.02*
Total Cholesterol (mmol/L)	4.10 (0.70)	4.19 (1.11)	4.03 (1.00)	0.76
Triglyceride (mmol/L)	1.09 ± 0.19	1.20 ± 0.25	1.17 ± 0.26	0.13
HDL-C(mmol/L)	1.42 ± 0.20	1.33 ± 0.22	1.33 ± 0.27	0.18
LDL-C(mmol/L)	2.50 ± 0.69	2.60 ± 0.69	2.64 ± 0.75	0.70

Data are presented as mean ± standard deviation or median (interquartile range). BMI, body mass index; AST, Aspartate Aminotransferase; ALT, Alanine Aminotransferase; HDL-C, High-Density Lipoprotein Cholesterol; LDL-C, Low-Density Lipoprotein Cholesterol. *P*-values from Kruskal-Wallis test or One-way ANOVA test, **P* < 0.05, ***P* < 0.01.

3.2 *Akkermansia muciniphila* PROBIO improved anthropometric parameters in overweight patients

After 8 weeks of intervention, body weight, BMI, and hip circumference in both the PB and POST groups showed a decrease compared to the PLA group, although the differences did not reach statistical significance (Table 2). Notably, after 8 weeks of intervention, waist circumference (Week 0 vs. Week 8 = 93.33 vs. 89.09, $p = 0.027$) and waist-to-hip ratio (Week 0 vs. Week 8 = 0.89 vs. 0.86, $p = 0.008$) were significantly reduced in the PB group, while no similar trend was observed in the POST group (Table 2). Further analysis revealed that, compared to the PLA group, the PB group exhibited significantly greater reductions in body weight ($P < 0.001$), fat mass ($P < 0.001$), and BMI ($P < 0.001$) (Fig. 2A-C). Meanwhile, the POST group also demonstrated significant reductions in body weight ($P < 0.01$), fat mass ($P < 0.01$), and BMI ($P < 0.01$). It is worth noting that although both viable *A. muciniphila* PROBIO and postbiotic interventions could reduce body weight, fat mass, and BMI in patients, the improvement in the PB group was significantly better than that in the POST group ($P < 0.05$). These results suggest that both viable *A. muciniphila* PROBIO and its postbiotics can effectively improve parameters such as body weight, fat mass, BMI, and waist-to-hip ratio in overweight patients, with viable *A. muciniphila* PROBIO demonstrating greater efficacy.

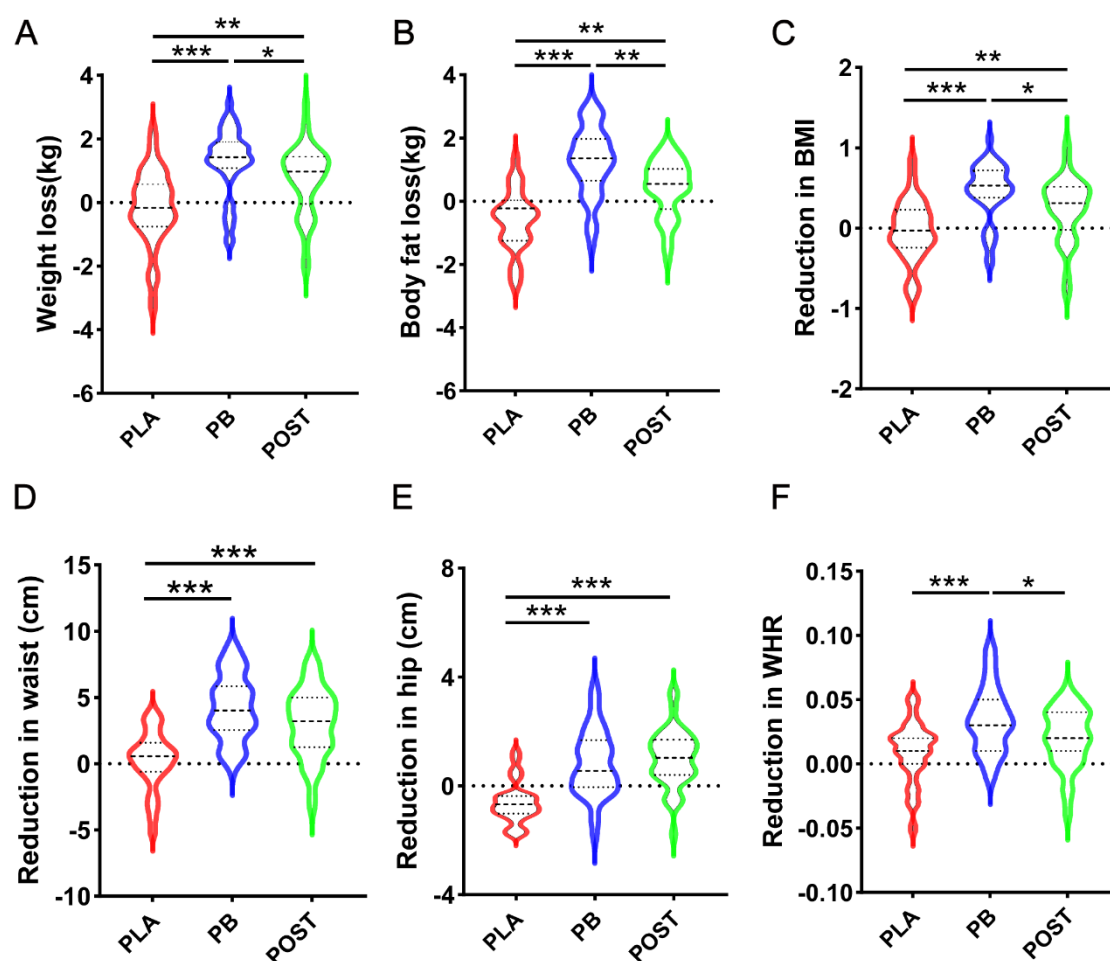


Fig. 2 Changes in body composition after eight-weeks treatment. (A) Changes in Body weight. (B) Changes in Fat mass. (C) Changes in BMI. (D) Changes in waist, (E) Changes in Hip, (F) Changes in WHR. PLA, (Placebo, $n = 30$); PB, (Probiotics, $n = 47$); POST, (Postbiotics, $n = 47$). All reduction or change metrics are calculated by 0-week values minus 8-week values. Statistical differences were assessed by Kruskal-Wallis test or One-way ANOVA test: * $P < 0.05$, ** $P < 0.001$ and *** $P < 0.001$.

Table 2 Changes in parameters associated with overweight from baseline after eight-weeks treatment

	PLA (n = 30)		P value	PB (n = 47)		P value	POST (n = 47)		P value
	Week 0	Week 8		Week 0	Week 8		Week 0	Week 8	
Body Weight (kg)	77.96 ± 9.30	78.11 ± 9.21	0.950	80.04 ± 10.9	78.74 ± 10.88	0.563	80.35 ± 10.18	79.62 ± 10.23	0.728
Fat mass (kg)	23.5 (2.77)	23.69 (2.33)	0.249	23.4 (8.00)	22.42 (7.10)	0.084	22.7 (5.90)	22.05 (5.49)	0.243
BMI (kg/m ²)	26.69 ± 1.63	26.72 ± 1.59	0.911	27.50 ± 3.06	27.04 ± 3.03	0.467	27.43 ± 2.24	27.18 ± 2.25	0.586
Waist (cm)	92.17 ± 6.87	91.89 ± 7.46	0.882	93.33 ± 9.29	89.09 ± 9.03	0.027*	93.46 ± 9.30	90.40 ± 8.94	0.107
Hip (cm)	102.89 ± 4.41	103.51 ± 4.27	0.580	101.78 ± 6.01	100.95 ± 5.79	0.496	103.3 ± 6.05	102.26 ± 5.86	0.402
Waist-hip ratio	0.89 (0.05)	0.88 (0.07)	0.544	0.89 (0.12)	0.86 (0.09)	0.008*	0.9(0.07)	0.88 (0.07)	0.089
HAMA-14a	14.33±5.65	14.17 ± 4.43	0.899	13.43 ± 5.26	8.26 ± 3.14	<0.001***	14.13 ± 5.32	9.79 ± 3.84	<0.001***
HDRS-17b	12.87±6.40	13.33 ± 5.88	0.770	11.81 ± 6.44	6.17 ± 3.67	<0.001***	12.72 ± 7.23	8.21 ± 3.54	<0.001***
PSQI	12.87 ± 4.55	11.87 ± 3.23	0.324	14.02 ± 4.22	10.32 ± 3.24	<0.001***	13.06 ± 4.48	9.43 ± 2.26	<0.001***
AST (U/L)	19.43 (9.30)	21.93(10.70)	0.408	23.42 (13.64)	21.74 (11.05)	0.199	21.37 (11.05)	19.26 (10.87)	0.661
ALT (U/L)	26.17 ± 10.01	26.89 ± 9.14	0.774	29.3 ± 11.8	26.10 ± 9.74	0.154	32.48 ± 12.23	27.13 ± 12.16	0.036*
Total Bilirubin (mg/dL)	0.57 ± 0.18	0.65 ± 0.20	0.114	0.68 ± 0.22	0.59 ± 0.17	0.031*	0.62 ± 0.19	0.68 ± 0.16	0.124
Direct Bilirubin (mg/dL)	0.26 ± 0.09	0.28 ± 0.15	0.480	0.29 ± 0.10	0.24 ± 0.09	0.012*	0.27 ± 0.07	0.27 ± 0.11	0.910
Indirect Bilirubin (mg/dL)	0.32 ± 0.22	0.37 ± 0.19	0.283	0.39 ± 0.18	0.35 ± 0.14	0.251	0.35 ± 0.20	0.40 ± 0.17	0.163
Total Cholesterol (mmol/L)	4.10 ± 0.52	4.34 ± 0.71	0.415	4.27 ± 0.74	3.95 ± 0.62	0.023*	4.24 ± 0.72	4.04 ± 0.63	0.175
Triglyceride (mmol/L)	1.09 ± 0.19	1.06 ± 0.17	0.494	1.20 ± 0.25	1.00 ± 0.32	<0.001***	1.17 ± 0.26	0.97 ± 0.25	<0.001***
HDL-C (mmol/L)	1.42 ± 0.20	1.34 ± 0.29	0.240	1.33 ± 0.22	1.36 ± 0.22	0.401	1.33 ± 0.27	1.34 ± 0.24	0.860
LDL-C (mmol/L)	2.50 ± 0.69	2.65 ± 0.68	0.410	2.60 ± 0.69	2.30 ± 0.72	0.044*	2.64 ± 0.75	2.54 ± 0.83	0.517

Data are presented as mean ± standard deviation or median (interquartile range). BMI, body mass index; AST, Aspartate Aminotransferase; ALT, Alanine Aminotransferase; HDL-C, High-Density Lipoprotein Cholesterol; LDL-C, Low-Density Lipoprotein Cholesterol. *P*-values from two-tailed unpaired T-test or two-tailed Mann-Whitney U test, **P* < 0.05, ***P* < 0.01, ****P* < 0.001

3.3 *Akkermansia muciniphila* PROBIO ameliorated hepatic function and lipid metabolic parameters in overweight patients

In terms of liver function improvement, an 8-week intervention with viable *A. muciniphila* PROBIO significantly reduced total bilirubin (Week 0 vs. Week 8 = 0.68 vs. 0.59, $p = 0.031$) and direct bilirubin levels (Week 0 vs. Week 8 = 0.29 vs. 0.24, $p = 0.012$) in overweight patients. Furthermore, eight weeks postbiotic intervention significantly downregulated ALT levels in overweight patients (Week 0 vs. Week 8 = 32.48 vs. 27.13, $p = 0.036$). Moreover, compared with the PLA group, the PB group showed significantly greater reduction in total bilirubin ($P < 0.05$) (Fig. 3A). The direct bilirubin levels in the PB group showed a similar trend (Fig. 3B). To evaluate lipid metabolism in each group, blood lipid parameters were evaluated across all three groups. After 8-week intervention with viable *A. muciniphila* PROBIO, subjects showed significant reduction in triglyceride levels (Week 0 vs. Week 8 = 1.20 vs. 1.00, $P < 0.001$). A similar trend was observed in the POST group, with significant decrease in blood triglycerides (Week 0 vs. Week 8 = 1.17 vs. 0.97, $P < 0.001$). Additionally, viable *A. muciniphila* PROBIO intervention significantly reduced total cholesterol levels (Week 0 vs. Week 8 = 4.27 vs. 3.95, $p = 0.023$). LDL-C levels were also significantly reduced after 8-week intervention with viable *A. muciniphila* PROBIO (Week 0 vs. Week 8 = 2.60 vs. 2.30, $p = 0.044$) (Table 2). Compared with the PLA group, the 8-week intervention with viable *A. muciniphila* PROBIO significantly enhanced the reduction of serum triglycerides ($P < 0.05$), total cholesterol ($P < 0.01$) and LDL-C ($P < 0.001$), while significantly attenuating the decrease of HDL-C ($P < 0.05$) (Fig. 3C-F). Comparatively, the POST group showed greater reductions in serum triglycerides ($P < 0.05$), total cholesterol ($P < 0.05$) and LDL-C levels ($P < 0.05$) than the PLA group, although these effects were pronounced than those observed in the PB group. Collectively, both viable *A. muciniphila* PROBIO and postbiotic interventions ameliorated lipid metabolic disorders in overweight patients, with superior efficacy observed in the PB group.

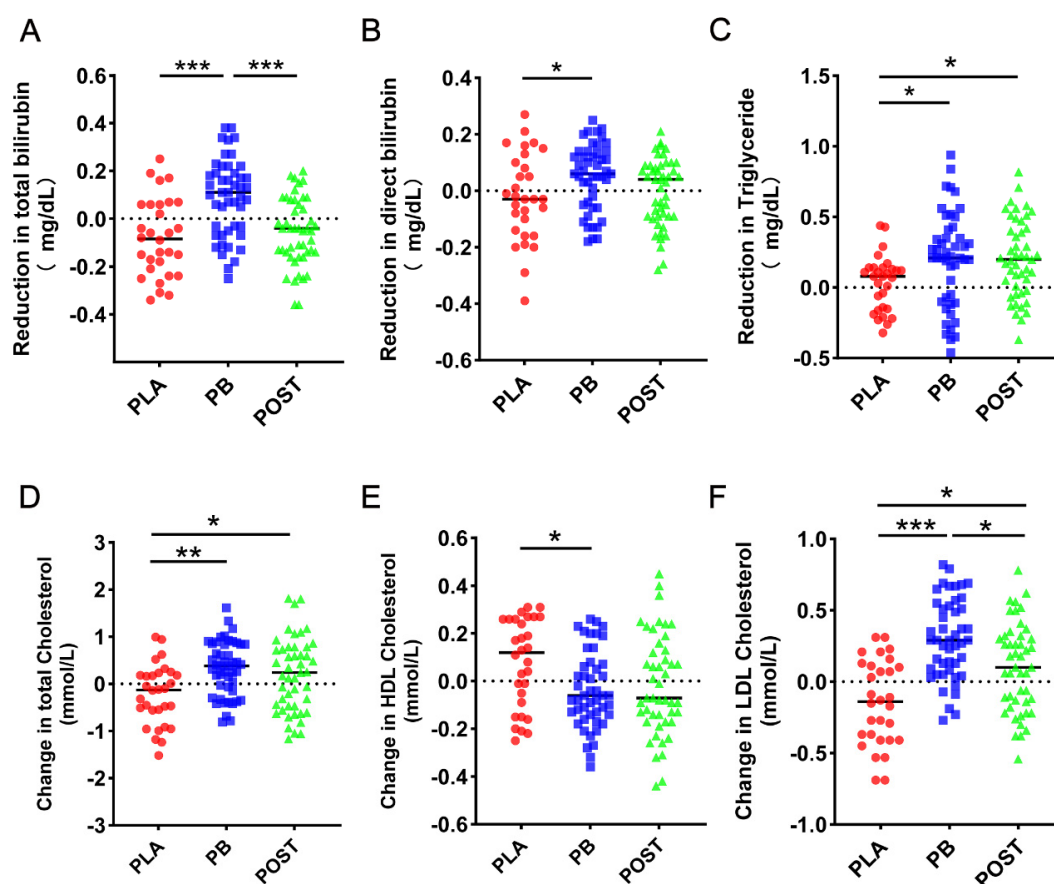


Fig. 3 Changes in liver function test and lipid metabolism parameters after eight-weeks treatment. (A-B) Changes in liver function test, total bilirubin(A), direct bilirubin (B). (C-F) Changes in lipid metabolic parameters, Triglyceride(C), Total cholesterol(D), HDL-C(E), and LDL-C(F). PLA, (Placebo, n = 30); PB, (Probiotics, n = 47); POST, (Postbiotics, n = 47). All reduction or change metrics are calculated by 0-week values minus 8-week values. Statistical differences were assessed by Kruskal-Wallis test or One-way ANOVA test: * $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$.

3.4 *Akkermansia muciniphila* PROBIO suppressed appetite and reduced inflammation in overweight patients

Overweight is frequently associated with imbalance of appetite-regulating hormones. In this study, changes in serum levels of GLP-1, PYY, and LEP were measured in patients receiving viable *A. muciniphila* PROBIO, postbiotics, or placebo. After the 8-week intervention, the PB group demonstrated statistically significant recovery in serum GLP-1 ($P < 0.05$), peptide YY (PYY, $P < 0.001$), and leptin (LEP, $P < 0.001$) concentrations. The POST group also showed marked recovery in the levels of GLP-1 ($P < 0.01$) and LEP ($P < 0.001$), but no significant change in PYY levels (Fig. 4A-C). Additionally, analysis of inflammatory factors revealed that IL-1 β ($P < 0.05$) in POST group and IL-6 ($P < 0.05$) in PB group were significantly decreased compared with PLA group (Fig. 4D-E). These findings indicate that both viable *A. muciniphila* PROBIO and its postbiotics effectively modulated appetite-regulating hormones and mitigated chronic inflammation, with the former demonstrating superior efficacy in regulating PYY and LEP, suggesting greater potential for clinical metabolic interventions.

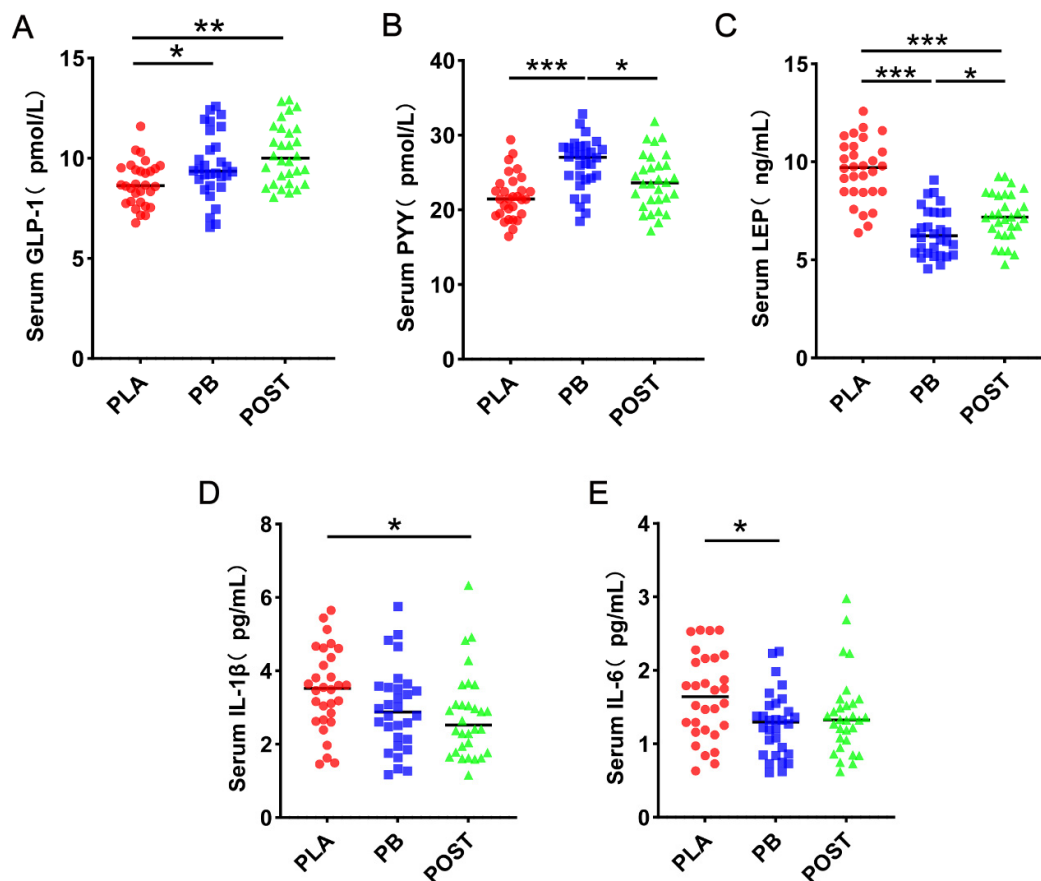


Fig. 4 Changes in metabolic hormones and inflammatory cytokines after eight-weeks treatment. (A-C) Changes in metabolic hormones, GLP-1(A), PYY(B), and LEP(C). (D-E) Changes in inflammatory cytokines, IL-1 β (D) and IL-6 (E).

PLA, (Placebo, n = 30); PB, (Probiotics, n = 47); POST, (Postbiotics, n = 47). Statistical differences were assessed by Kruskal-Wallis test or One-way ANOVA test: * $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$.

3.5 *Akkermansia muciniphila* PROBIO alleviated anxiety, depression, and sleep disturbances in overweight patients

To evaluate mood and sleep status in overweight subjects, a comprehensive assessment was conducted in all groups before and after the intervention by using the HAMA-14, the HDRS-17, and the PSQI. After 8-week intervention, the probiotic group showed significant reduction in HAMA-14 anxiety scores (Week 0 vs. Week 8 = 13.43 vs. 8.26, $P < 0.001$), indicating improved anxiety symptoms (Table 2). The POST group also showed significant decrease in anxiety scores (Week 0 vs. Week 8 = 14.13 vs. 9.79, $P < 0.001$). Moreover, HDRS-17 depression scores showed both probiotic ($P < 0.001$) and postbiotic ($P < 0.001$) interventions significantly alleviated depressive symptoms (Table 2). PSQI results indicated that 8-week viable *A. muciniphila* PROBIO intervention significantly improved sleep quality (Week 0 vs. Week 8 = 14.02 vs. 10.32, $P < 0.001$), and postbiotic intervention for 8 weeks also significantly improved sleep quality (Week 0 vs. Week 8 = 13.06 vs. 9.43, $P < 0.001$) (Table 2). As shown in Fig 5 A-C, improvements in anxiety, depression and insomnia were significantly greater in PB or POST groups compared with PLA group. To investigate potential mechanisms of mood improvement, we measured serum 5-HT and DA levels by ELISA. Results showed probiotic intervention significantly increased serum 5-HT (placebo vs. probiotic = 135.41 vs. 161.35, $p = 0.049$), with similar trend in postbiotic group ($P < 0.05$) (Fig. 5E). Meanwhile, serum DA levels were

significantly restored in PB group ($P < 0.05$), whereas postbiotic treatment failed to demonstrate significant DA modulation (Fig. 5F). These results suggest that both viable *A. muciniphila* PROBIO and postbiotics improved emotional disorders such as anxiety, depression and insomnia in overweight patients.

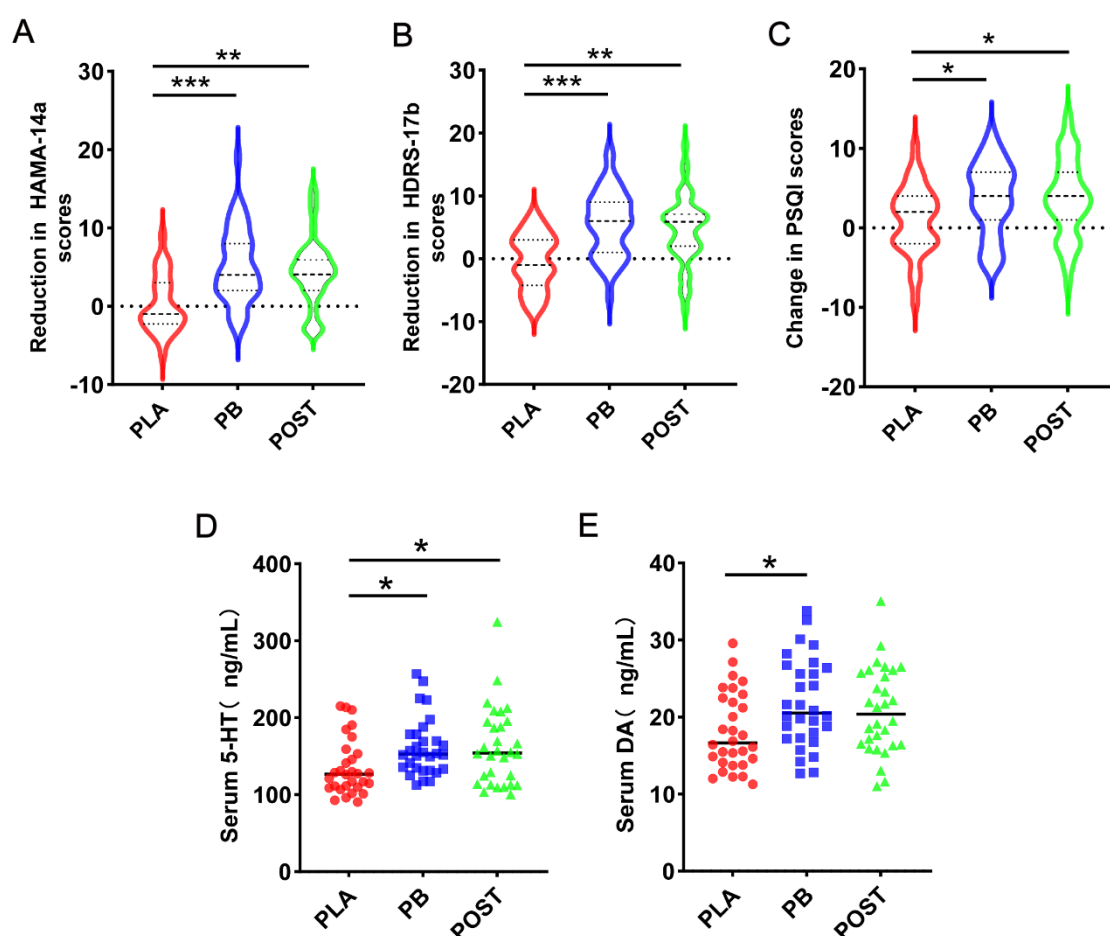


Fig. 5 Changes in anxiety and depression-like behavior parameters and neurotransmitters after eight-weeks treatment. (A) Changes in HAMA-14a scores. (B) Changes in HDRS-17b scores. (C) Changes in PSQI scores. (D-E) Levels of serum 5-HT(D), and serum DA(E) after eight-weeks treatment. PLA, (Placebo, n = 30); PB, (Probiotics, n = 47); POST, (Postbiotics, n = 47). All reduction or change metrics are calculated by 0-week values minus 8-week values. Statistical differences were assessed by Kruskal-Wallis test or One-way ANOVA test: * $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$.

3.6 *Akkermansia muciniphila* PROBIO restored the diversity of gut microbiota in overweight patients

To investigate the effects of viable *A. muciniphila* PROBIO and its postbiotics on gut microbiota diversity, we analyzed gut microbiota of participants from normal people (HP) group, PLA group, PB group and POST group using 16S high-throughput sequencing. α -diversity analysis showed that compared with PLA group, PB group had significantly restored CHAO1 index ($P < 0.01$), Shannon index ($P < 0.01$) and Simpson index ($P < 0.01$) to HP group levels (Fig. 6A-C). POST group also showed significant improvement in Shannon index ($P < 0.05$) and Simpson index ($P < 0.01$) compared with PLA group (Fig. 6A-C). PCoA analysis indicated that gut microbiota structure in PB and POST groups differed from PLA group (Fig. 6D). Venn diagram showed significantly increased microbial diversity in PB group but not in POST group (Fig. 6E). LEfSe analysis indicated both viable *A. muciniphila* PROBIO and postbiotic interventions significantly improved gut microbiota structure in overweight patients (Fig. 6F).

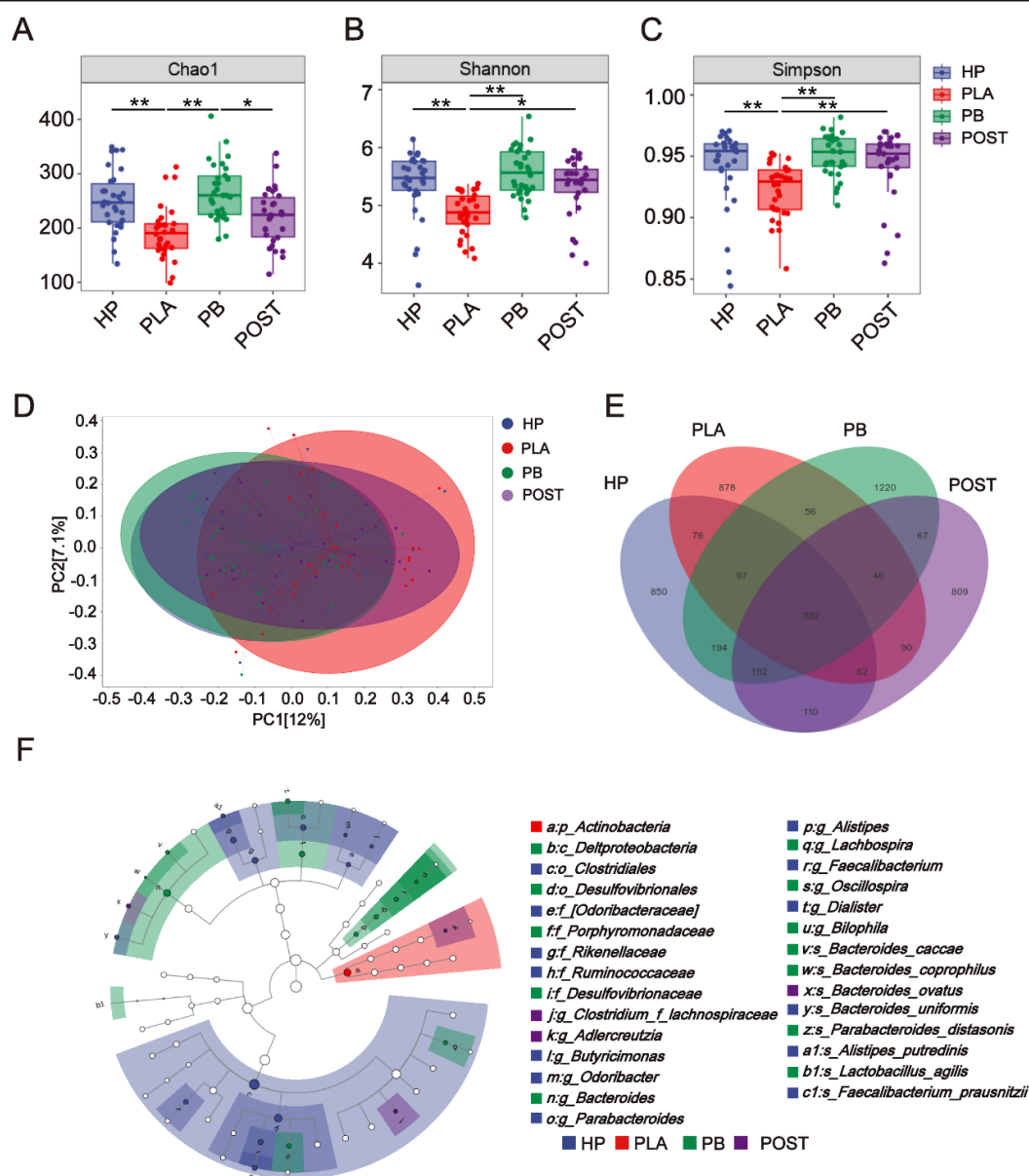


Fig. 6 *Akkermansia muciniphila* improved the diversity of gut microbiota in patients with overweight or obesity. (A) The Chao1 index. (B) The Shannon index. (C) The Simpson index. (D) Beta diversity was assessed through PCoA. (E) The Venn diagram depicted the overlap of OTUs. (F) LDA Effect Size analysis. HP, (Noraml people, n = 30); PLA, (Placebo, n = 30); PB, (Probiotics, n = 30); POST, (Postbiotics, n = 30). Statistical differences were assessed by Kruskal-Wallis test or One-way ANOVA test: * $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$.

3.7 *Akkermansia muciniphila* PROBIO restored the composition of gut microbiota in overweight patients

To investigate the effects of viable *A. muciniphila* PROBIO and postbiotics on gut microbiota structure, we analyzed phylum-level composition of gut microbiota in each group (Fig. 7A). Results showed that relative abundances of Firmicutes and Bacteroidetes in PB and POST groups recovered to HP group levels (Fig. 7B-C). Both the PB and POST groups exhibited decreased relative abundances of Proteobacteria and Actinobacteria compared with the PLA group (Fig. 7D-E). Notably, the relative abundance of Verrucomicrobia in PB group significantly recovered to levels of the HP group ($P < 0.01$) and was significantly higher than POST group ($P < 0.05$) (Fig. 7F). No significant differences in the relative abundance of Fusobacteria were observed among four groups (Fig. 7G).

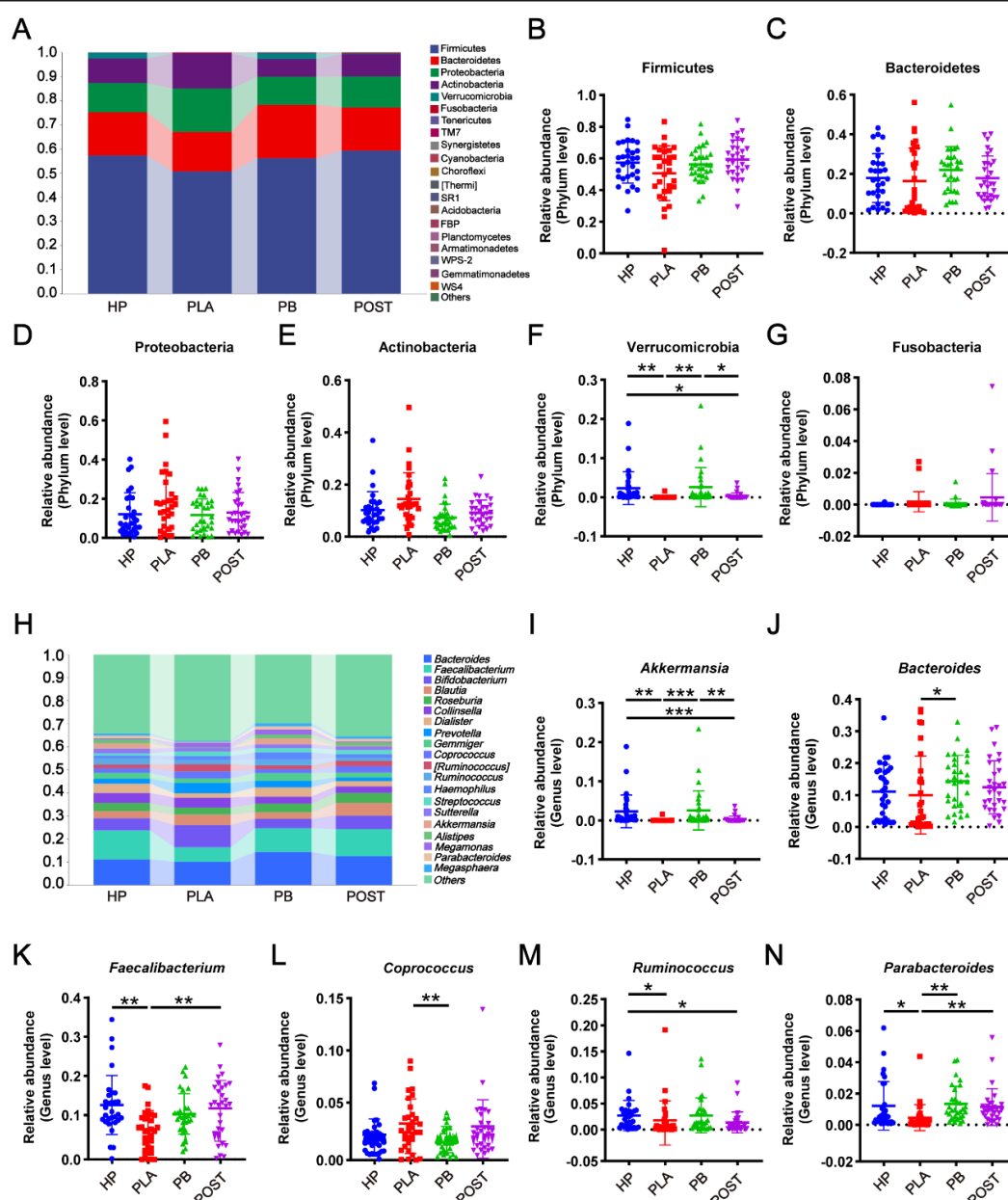


Fig. 7 *Akkermansia muciniphila* altered gut microbiota composition in patients with overweight. (A) The distribution of the gut microbiota at the phylum level. (B–G) The relative abundance of the Firmicutes (B), Bacteroidetes (C), Proteobacteria (D), Actinobacteria (E), Verrucomicrobia (F), and Fusobacteria (G). (H) Genus level distribution of the gut microbiota. (I–N) The relative abundance of *Akkermansia* (I), *Bacteroides* (J), *Faecalibacterium* (K), *Coprococcus* (L), *Ruminococcus* (M), and *Parabacteroides* (N). HP, (Noraml people, n = 30); PLA, (Placebo, n = 30); PB, (Probiotics, n = 30); POST, (Postbiotics, n = 30). Statistical differences were assessed by Kruskal-Wallis test or One-way ANOVA test: * $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$.

Taxonomic analysis at genus level demonstrated PB group exhibited a significant increase in the relative abundance of *Akkermansia* to HP group levels ($P < 0.001$), while POST group showed no change (Fig. 7I). Moreover, A significant increase was also observed in the PB group for the relative abundance of *Bacteroides* ($P < 0.05$) (Fig. 7J). And there is a significant upregulation of *Faecalibacterium* in POST group, while no significant increase in PB group (Fig. 7K). Intervention with viable *A. muciniphila* PROBIO led to a significant reduction in *Coprococcus* levels ($P < 0.01$), with no such effect observed in POST group (Fig. 7L). Additionally, the levels of *Ruminococcus* in PB group were restored to HP group levels, although not reaching statistical significance (Fig. 7M). Both PB and POST groups showed significant upregulation in the relative

abundance of *Parabacteroides* ($P < 0.01$), reaching the level of HP group (Fig. 7N). These results indicated that viable *A. muciniphila* PROBIO is superior to its postbiotics in modulating gut microbiota composition.

3.8 Correlation analysis between gut microbiota and clinical parameters related to overweight

Spearman correlation analysis was adopted to evaluate the relationships between gut microbial composition and overweight-related clinical parameters, including changes in body weight, body fat, waist circumference changes, mood status and serum lipid metabolism parameter. The results demonstrated that the relative abundance of *A. muciniphila* PROBIO was significantly positively correlated with weight loss, fat reduction, waist circumference reduction, improvements in anxiety/depression, sleep quality enhancement and improved lipid metabolism. The relative abundance of *Parabacteroides* showed significant positive correlations with fat reduction, waist circumference reduction, mood improvement and LDL-C reduction. Additionally, the relative abundance of *Faecalibacterium* was positively associated with anxiety relief, improvements in total cholesterol and LDL-C levels. Conversely, the relative abundance of *Coprococcus* was negatively correlated with improvements in anxiety/depression and LDL-C (Fig. 8).

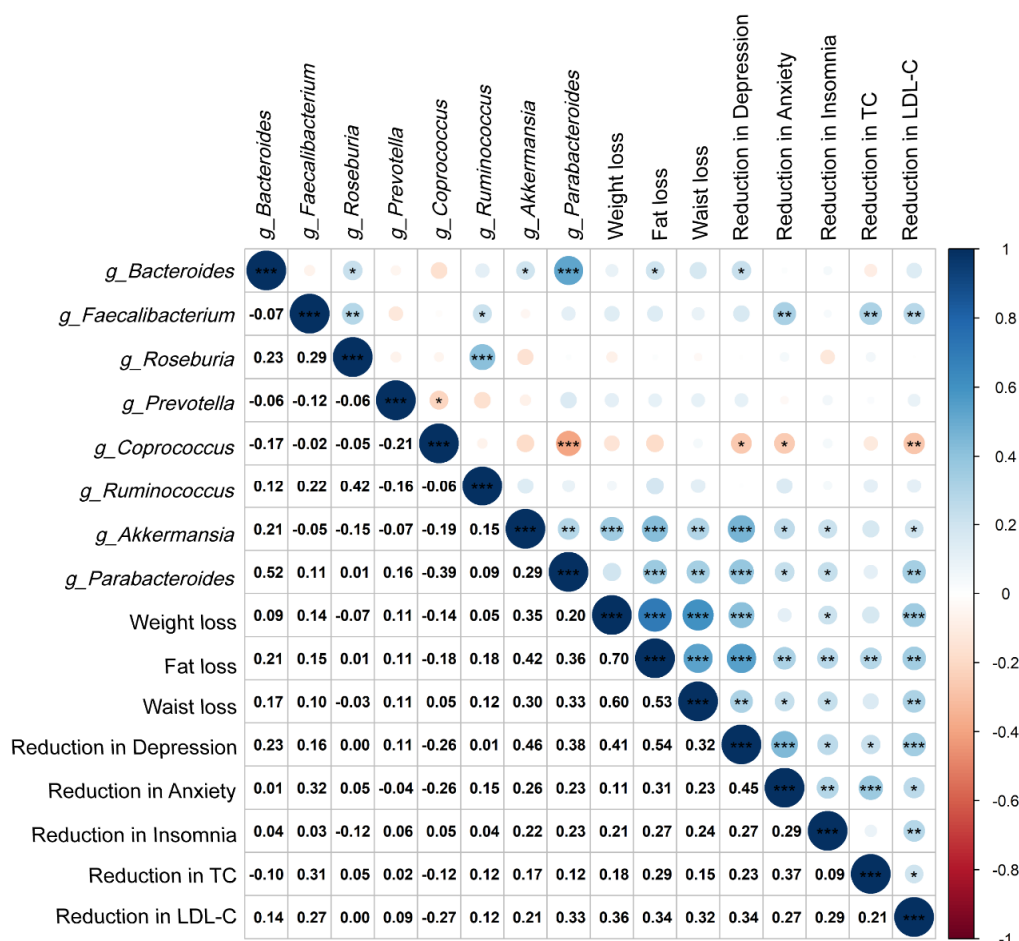


Fig. 8 Correlation heatmap analysis of gut microbiota and overweight parameters using Spearman's rank correlation. Blue indicates a positive correlation, while red represents a negative correlation. * $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$.

4. Discussion

Overweight has been recognized as a common systemic metabolic disorder closely associated with the gut microbiota^[19]. Probiotics, as effective modulators of the gut microbiota, have demonstrated significant

beneficial effects on various metabolic diseases, including obesity, type 2 diabetes, hypertension, non-alcoholic fatty liver disease (NAFLD) and insulin resistance, thus being considered as potential strategy for alleviating obesity or overweight^[20]. *A. muciniphila*, a gut resident bacterium, can colonize the intestinal mucus layer and exert important protective effects on host health through multiple mechanisms, including the production of beneficial metabolites, enhancement of gut barrier function, regulation of immune responses, and improvement of energy metabolism^[15]. Previous studies have confirmed that *A. muciniphila* supplementation significantly reduced body weight and fat in overweight patients, improved metabolic blood parameters, and alleviated accompanying anxiety and depressive symptoms.

In this study, following an 8-week intervention with viable *A. muciniphila* PROBIO and its postbiotics, overweight patients exhibited reductions in body weight, fat mass and BMI compared to baseline, but without significant difference compared with placebo group, which may be related to the short intervention period (Table 2). However, viable *A. muciniphila* PROBIO intervention significantly reduced waist circumference and waist-hip ratio, while its postbiotics showed no such effects (Table 2). This result is consistent with previous clinical studies showing that AKK-WST01 supplementation reduced body weight and visceral/total fat mass in overweight/obese T2DM patients^[18]. In contrast to previous studies, the viable *A. muciniphila* PROBIO group in our research demonstrated greater improvements in body weight, fat mass, and BMI compared to the postbiotic group (Fig. 2).

Accumulating evidence indicates that overweight individuals commonly exhibit impaired liver function, metabolic disturbances, and persistent low-grade inflammation.^[21, 22] An animal study demonstrated that *A. muciniphila* could ameliorate metabolic dysfunction-associated fatty liver disease by regulating L-aspartate metabolism and lipid oxidation through the gut-liver axis^[23]. Furthermore, *A. muciniphila* has been shown to prevent non-alcoholic steatohepatitis by regulating $\gamma\delta$ T17 cells and macrophage polarization^[24]. These findings suggest that *A. muciniphila* may possess regulatory potential for regulating liver function. In this study, although interventions with viable *A. muciniphila* PROBIO and its postbiotics did not significantly restore serum AST and ALT levels in patients, the viable bacteria intervention significantly improved total and direct bilirubin levels, while no similar effect was observed in the postbiotic group, further confirming its regulatory and protective effects on liver function (Table 2). Moreover, the improvement effects of viable *A. muciniphila* PROBIO on reduction in total and direct bilirubin were superior to those of the *A. muciniphila* PROBIO postbiotic group (Fig. 3 A-B).

Overweight is frequently associated with dyslipidemia, thereby increasing the risk of metabolic and cardiovascular diseases^[25]. To evaluate cardiovascular disease risk in overweight individuals, blood levels of total cholesterol, LDL-C, and HDL-C were measured. Both viable *A. muciniphila* PROBIO and postbiotic interventions significantly reduced triglyceride levels (Table 2). Furthermore, viable *A. muciniphila* PROBIO showed superior improvement in lipid metabolism compared to *A. muciniphila* PROBIO postbiotics in overweight patients (Fig. 3 C-F). These findings are consistent with those of Zhang et al., who reported that *A. muciniphila* PROBIO may facilitate the alleviation of overweight by enhancing lipid metabolism^[18].

Animal studies also indicated that *A. muciniphila* PROBIO alleviates hyperlipidemia by regulating lipid metabolism and immune function in APOE*3-Leiden.CETP mice.

It has been well documented that obesity is associated with reduced GLP-1 and PYY secretion, which plays a crucial role in the disruption of normal appetite regulation^[26]. In this study, both viable *A. muciniphila* PROBIO and pasteurized *A. muciniphila* significantly improved serum levels of GLP-1, PYY, and LEP in patients (Fig. 4A-C). Mechanistically, *A. muciniphila* significantly enhances butyrate production in the gut through its metabolic activity. The resultant butyrate directly modulates intestinal L cells, thereby stimulating the secretion of GLP-1 and PYY^[27, 28]. Another animal study revealed that *A. muciniphila* supplementation significantly inhibited TNF- α -induced JNK-dependent IRS-1 serine phosphorylation in diet-induced obesity models, thereby improving GLP-1 signaling efficiency^[29]. In overweight patients, excessive adipose tissue accumulation leads to adipocyte hypertrophy and dysfunction, which in turn results in the release of large quantities of pro-inflammatory cytokines that contribute to systemic inflammation^[22]. Previous studies have demonstrated that *A. muciniphila* PROBIO can alleviate intestinal inflammation by downregulating the expression of PI3K upstream receptor gene^[30]. In the present study, ELISA results in this study revealed that both viable *A. muciniphila* PROBIO and postbiotics significantly reduced serum IL-6 and IL-1 β levels in patients, thereby demonstrating anti-inflammatory effects (Fig.4 D-E). These findings suggest that *A. muciniphila* PROBIO may serve as a promising therapeutic agent for managing overweight and associated metabolic dysfunctions, with viable bacteria exhibiting more pronounced efficacy.

Obesity and overweight have been shown to contribute to the onset and worsening of depressive and anxiety disorders, indicating a bidirectional interaction between metabolic and mental health conditions^[4]. A nationwide Swedish survey revealed that obesity increases the risk of anxiety or depression in children and adolescents by 43%^[31]. Previous studies found that high-fat diet-induced obesity in mice exacerbates depression- and anxiety-like behaviors through AMPK/mTOR signaling pathway-mediated autophagy^[32]. In our study, we observed that both viable *A. muciniphila* PROBIO and its postbiotics significantly improved anxiety, depression and sleep conditions in overweight individuals (Table 2). Moreover, both viable *A. muciniphila* PROBIO and its postbiotics improved anxiety, depression, and insomnia in overweight patients (Fig. 5A-C). Additionally, the viable *A. muciniphila* PROBIO group showed significantly increased levels of mood-related biomarkers 5-HT and DA in blood, while its postbiotics also elevated blood 5-HT levels (Fig. 5E-F). As a neurotransmitter, 5-HT can ameliorate depressive and anxiety symptoms by modulating emotion-related brain regions like the amygdala and enhancing neuroplasticity^[33]. These results suggest that *A. muciniphila* PROBIO and its postbiotics may alleviate overweight-associated anxiety and depression by regulating gut-derived tryptophan metabolites, including 5-HT and DA. Previous studies have confirmed that *A. muciniphila* can improve anxiety and depression through multiple pathways. Oral administration of *A. muciniphila* can reverse chronic stress-induced depressive behaviors and normalize abnormal 5-HT metabolism in both brain and gut^[34]. Moreover, the outer membrane protein Amuc_1100 and extracellular vesicles from *A. muciniphila* have been proven to alleviate anxiety- and depression-like behaviors^[35].

Meanwhile, *A. muciniphila* can significantly increase the abundance of beneficial bacteria while reducing the proliferation of harmful bacteria in the gut. This microbial remodeling is closely related to the improvement of anxiety and depressive behaviors^[35, 36]. For example, enrichment of *A. muciniphila* can promote the production of indole-3-lactic acid, thereby regulating lipid metabolism and expression of mood-related genes^[37].

A growing body of evidence indicated that gut microbiota played a critical regulatory role in overweight and associated anxiety/depression. Overweight-induced chronic inflammation and immune dysregulation damage the intestinal barrier, leading to gut microbiota dysbiosis, which subsequently triggers metabolic disorders and neurological abnormalities^[38, 39]. Studies have revealed a reduction in the diversity and compositional disorder of gut microbiota in overweight individuals^[38]. To investigate whether *A. muciniphila* PROBIO can ameliorate overweight -induced gut microbiota imbalance, 16s high-throughput sequencing on fecal microbiota of patients was conducted. The results showed that viable *A. muciniphila* PROBIO and its postbiotic interventions significantly improved α -diversity and β -diversity of gut microbiota in overweight patients (Fig. 6A-E). LEfSe analysis and Venn diagrams further demonstrated significant differences in gut microbiota composition across normal, placebo, postbiotic, and probiotic groups (Fig. 6F-G). Consistent with Clara Depommier et al.'s study, both viable and inactivated *A. muciniphila* PROBIO interventions effectively modulated gut microbiota structure in overweight/obese patients, thereby ameliorating disease phenotypes^[16].

At the phylum level, we observed an increase in the relative abundance of Firmicutes and Bacteroidetes, alongside a decrease in relative abundance of Actinobacteriota and Proteobacteria in the gut microbiota of patients following intervention with viable *A. muciniphila* PROBIO or its postbiotics, although the differences were not statistically significant (Fig. 7A-G). Proteobacteria includes various pathogenic genera such as *Salmonella*, *Shigella*, and *Klebsiella*, whose infections may lead to severe diseases including sepsis, gastrointestinal infections, diarrhea, and urinary tract infections^[40, 41]. Bacteroidetes, a major component of human gut microbiota, contains several beneficial genera such as *Bacteroides* and *Prevotella*. The studies have shown that *Bacteroides* significantly ameliorated high-fat diet-induced weight gain, metabolic dysfunction and anxiety-like behaviors in mice, suggesting *Bacteroides* may have therapeutic potential for metabolic disorders such as overweight^[42].

At the genus level, oral administration of viable *A. muciniphila* significantly increased the relative abundance of *Akkermansia*, *Bacteroides*, and *Parabacteroides* while decreasing *Coprococcus* in gut microbiota. Postbiotic intervention with *A. muciniphila* PROBIO also significantly upregulated the relative abundance of *Faecalibacterium* and *Parabacteroides* in the gut microbiota (Fig. 7H-N). An animal study demonstrated that dietary fiber supplementation improved host metabolism and alleviated dyslipidemia by enriching beneficial bacteria like *Ruminococcus* and *Akkermansia*, thereby counteracting obesity^[43]. Another study found reduced abundance of *Faecalibacterium* in patients with active Crohn's disease^[44]. As an important probiotic genus, *Parabacteroides distasonis* reduces hyperlipidemia by metabolizing bile acids to produce succinate, activating FXR pathway, and restoring intestinal barrier integrity^[45]. *Akkermansia*, as a

promising next-generation probiotic, has been shown to enhance gut barrier function and suppress inflammation, contributing to overall host health^[46]. In summary, *A. muciniphila* PROBIO intervention can modulate the diversity and composition of gut microbiota in overweight patients, which may underlie its anti-overweight effects.

Although this study provides a natural, safe and effective therapeutic strategy for overweight, there are still several issues worthy of further investigation. An 8-week intervention period was selected due to low patient compliance in clinical studies, which is relatively short compared to other obesity-related trials and may not fully reflect the therapeutic efficacy of *A. muciniphila* PROBIO. Moreover, the single-center design and small sample size may have resulted in the loss of potentially valuable clinical data. Our data show that viable *A. muciniphila* PROBIO is more advantageous than pasteurized *A. muciniphila* PROBIO in improving overweight, which differs from the results reported by Clara Depommier et al. showing that inactivated *A. muciniphila* is superior in weight control, lipid metabolism regulation and anti-inflammatory effects. These divergent observations may be attributed to the following underlying mechanisms: Primarily, different *A. muciniphila* strains exhibit variations in functional components, which can lead to divergent therapeutic outcomes. Moreover, viable *A. muciniphila* possess sustained mucolytic activity^[47], enabling continuous generation of short-chain fatty acids (e.g., acetate and propionate) that directly modulate host energy homeostasis and mediate anti-inflammatory responses^[48, 49]. Furthermore, viable *A. muciniphila* demonstrate superior efficacy in remodeling gut microbial architecture, characterized by diminished abundance of pro-inflammatory taxa (e.g., *Tyzzereella*) and enriched populations of beneficial species (e.g., *Ruminiclostridium*)^[47]. Conversely, inactivated *A. muciniphila* exhibit constrained microbiota-modulating capacity due to their impaired colonization competence. Additionally, viable *A. muciniphila* maintain intestinal mucosal integrity through mucin degradation pathway and can upregulate tight junction protein expression^[50]. Current studies have not confirmed that pasteurized *A. muciniphila* possess intestinal barrier repair function of equal strength. These findings further support the conclusion that viable *A. muciniphila* demonstrates superior efficacy compared to pasteurized *A. muciniphila* in improving metabolic disorders such as overweight. Importantly, our study lacks pre-intervention assessment of baseline *A. muciniphila* abundance in each group, which may lead to the omission of some scientifically valuable experimental results. Previous studies have shown that *A. muciniphila* supplementation only shows significant weight loss and metabolic regulation effects in subjects with low baseline *A. muciniphila* abundance^[16]. Consequently, we propose to establish a well-characterized overweight cohort in subsequent investigations to systematically evaluate the association between pre-intervention bacterial abundance and therapeutic efficacy. And there are inherent limitations in our reliance on self-reported lifestyle investigation without quantitative dietary records or objective physical activity monitoring. Although participants were thoroughly instructed to maintain their habitual lifestyle and compliance was verbally confirmed during weekly check-ins, objective measurements would have provided more rigorous documentation. Future investigations on overweight will implement standardized nutritional evaluations and triaxial accelerometry to rigorously account for lifestyle-related

confounders. Moreover, although the sample size of the PLA group in this study met the minimum statistical requirements, the relatively small cohort (n=30) may have introduced several methodological constraints, potentially leading to bias and obscuring true treatment effects.

5. Conclusion

This study systematically demonstrated that both viable *A. muciniphila* PROBIO and its postbiotic preparation effectively reduced overweight-related parameters, including body weight and fat mass, in overweight patients (Fig. 9). In addition, both interventions significantly alleviated anxiety and depression symptoms and improved sleep quality. Moreover, *A. muciniphila* PROBIO intervention ameliorated hepatic dysfunction and lipid metabolism disorders in patients. Notably, the viable *A. muciniphila* PROBIO formulation exhibited superior efficacy over its postbiotics in ameliorating both overweight conditions and mood disorders. Mechanistic investigations further suggested that *A. muciniphila* PROBIO may exert its multifaceted regulatory effects on metabolic disorders and emotional problems by remodeling gut microbiota structure and diversity.

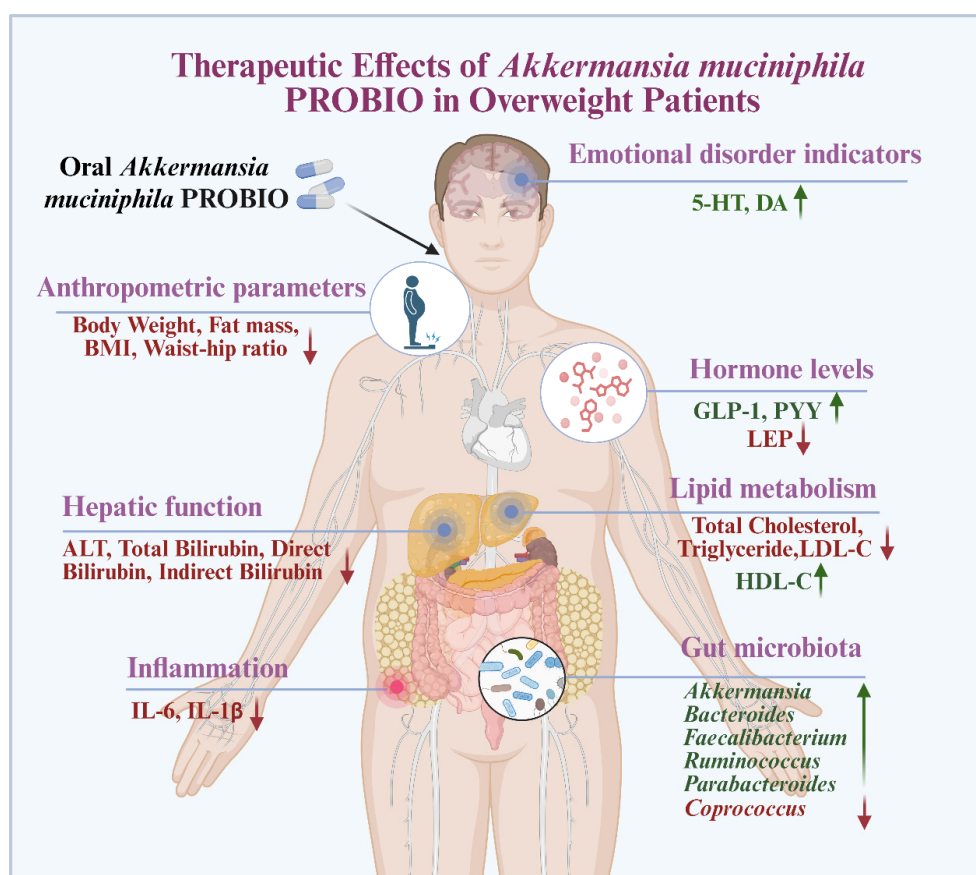


Fig. 9 Diagram of the potential mechanism by which *Akkermansia muciniphila* PROBIO ameliorates overweight. Oral administration of viable *A. muciniphila* and its postbiotics ameliorated overweight-associated metabolic disorders through multi-target mechanisms, including reducing lipid accumulation, modulating liver function, restoring hormonal levels, suppressing inflammation, and remodeling gut microbiota.

Declaration of Interest Statement

There are no conflicts to declare.

Acknowledgements

This research received funding from the National Natural Science Foundation of China (Grant No. 82060638 and 82460297 to Tingtao Chen). Furthermore, financial backing for this study was also provided by the Double Thousand Plan of Jiangxi Province to Tingtao Chen (High-End Talents Project of Scientific and Technological Innovation). Additionally, this research was supported by the Jiangxi Province Key Laboratory of bioengineering drugs (Reference number 2024SSY07061).

Data Availability Statement

Raw 16S rDNA sequencing data from this study have been deposited at NCBI SRA Biological Project No. PRJNA1251145.

Ethical Statements

The studies were approved by the Medical Ethics Committee of Nanchang Reproductive Hospital (Approval No. [2024] 026)

References

- [1] B. Wang, Q. Kong, S. Cui, et al., Bifidobacterium adolescentis Isolated from Different Hosts Modifies the Intestinal Microbiota and Displays Differential Metabolic and Immunomodulatory Properties in Mice Fed a High-Fat Diet, *Nutrients*. 13 (2021) <https://doi.org/10.3390/nu13031017>.
- [2] L. Maretty, D. Gill, L. Simonsen, et al., Proteomic changes upon treatment with semaglutide in individuals with obesity, *Nat Med*. 31 (2025) 267-277. <https://doi.org/10.1038/s41591-024-03355-2>.
- [3] A. Zarzour, H.W. Kim, N.L. Weintraub, Understanding Obesity-Related Cardiovascular Disease: It's All About Balance, *Circulation*. 138 (2018) 64-66. <https://doi.org/10.1161/CIRCULATIONAHA.118.034454>.
- [4] S. Fulton, L. Decarie-Spain, X. Fioramonti, et al., The menace of obesity to depression and anxiety prevalence, *Trends Endocrinol Metab*. 33 (2022) 18-35. <https://doi.org/10.1016/j.tem.2021.10.005>.
- [5] M. Solas, F.I. Milagro, D. Martinez-Urbistondo, et al., Precision Obesity Treatments Including Pharmacogenetic and Nutrigenetic Approaches, *Trends Pharmacol Sci*. 37 (2016) 575-593. <https://doi.org/10.1016/j.tips.2016.04.008>.
- [6] H. Yang, W. Lan, C. Luo, et al., Lactobacillus plantarum 24-7 improves postoperative bloating and hard stools by modulating intestinal microbiota in patients with congenital heart disease: a randomized controlled trial, *Food Funct*. 15 (2024) 2090-2102. <https://doi.org/10.1039/d3fo05452g>.
- [7] Q. Wang, J. Xiong, Y. He, et al., Effect of L-arabinose and lactulose combined with Lactobacillus plantarum on obesity induced by a high-fat diet in mice, *Food Funct*. 15 (2024) 5073-5087. <https://doi.org/10.1039/d4fo00369a>.
- [8] C. Zhang, R. Fang, X. Lu, et al., Lactobacillus reuteri J1 prevents obesity by altering the gut microbiota and regulating bile acid metabolism in obese mice, *Food Funct*. 13 (2022) 6688-6701. <https://doi.org/10.1039/d1fo04387k>.
- [9] T. Wang, J. Han, H. Dai, et al., Polysaccharides from *Lyophyllum decastes* reduce obesity by altering gut microbiota and increasing energy expenditure, *Carbohydr Polym*. 295 (2022) 119862. <https://doi.org/10.1016/j.carbpol.2022.119862>.
- [10] D.D. Heeney, Z. Zhai, Z. Bendiks, et al., Lactobacillus plantarum bacteriocin is associated with intestinal and systemic improvements in diet-induced obese mice and maintains epithelial barrier integrity in vitro, *Gut Microbes*. 10 (2019) 382-397. <https://doi.org/10.1080/19490976.2018.1534513>.
- [11] Q. Chen, C. Mei, M. Guo, et al., Lactobacillus plantarum CCFM1180 attenuates obesity induced by estrogen deficiency by activating estrogen receptor alpha in abdominal adipose tissue and regulating gut microbiota-derived metabolites, *Food Science and Human Wellness*. 13 (2024) 765-777. <https://doi.org/10.26599/fshw.2022.9250065>.

- [12] Y.G. Cho, Y.J. Yang, Y.S. Yoon, et al., Effect of MED-02 Containing Two Probiotic Strains, *Limosilactobacillus fermentum* MG4231 and MG4244, on Body Fat Reduction in Overweight or Obese Subjects: A Randomized, Multicenter, Double-Blind, Placebo-Controlled Study, *Nutrients*. 14 (2022) <https://doi.org/10.3390/nu14173583>.
- [13] M. Sohn, G.Y. Na, J. Chu, et al., Efficacy and Safety of *Lactobacillus plantarum* K50 on Lipids in Koreans With Obesity: A Randomized, Double-Blind Controlled Clinical Trial, *Front Endocrinol (Lausanne)*. 12 (2021) 790046. <https://doi.org/10.3389/fendo.2021.790046>.
- [14] A. Solito, N. Bozzi Cionci, M. Calgaro, et al., Supplementation with *Bifidobacterium breve* BR03 and B632 strains improved insulin sensitivity in children and adolescents with obesity in a cross-over, randomized double-blind placebo-controlled trial, *Clin Nutr*. 40 (2021) 4585-4594. <https://doi.org/10.1016/j.clnu.2021.06.002>.
- [15] R. Ghotaslou, E. Nabizadeh, M.Y. Memar, et al., The metabolic, protective, and immune functions of *Akkermansia muciniphila*, *Microbiol Res*. 266 (2023) 127245. <https://doi.org/10.1016/j.micres.2022.127245>.
- [16] C. Depommier, A. Everard, C. Druart, et al., Supplementation with *Akkermansia muciniphila* in overweight and obese human volunteers: a proof-of-concept exploratory study, *Nat Med*. 25 (2019) 1096-1103. <https://doi.org/10.1038/s41591-019-0495-2>.
- [17] C. Depommier, M. Van Hul, A. Everard, et al., Pasteurized *Akkermansia muciniphila* increases whole-body energy expenditure and fecal energy excretion in diet-induced obese mice, *Gut Microbes*. 11 (2020) 1231-1245. <https://doi.org/10.1080/19490976.2020.1737307>.
- [18] Y. Zhang, R. Liu, Y. Chen, et al., *Akkermansia muciniphila* supplementation in patients with overweight/obese type 2 diabetes: Efficacy depends on its baseline levels in the gut, *Cell Metab*. 37 (2025) 592-605 e596. <https://doi.org/10.1016/j.cmet.2024.12.010>.
- [19] A.C. Nicolucci, M.P. Hume, I. Martinez, et al., Prebiotics Reduce Body Fat and Alter Intestinal Microbiota in Children Who Are Overweight or With Obesity, *Gastroenterology*. 153 (2017) 711-722. <https://doi.org/10.1053/j.gastro.2017.05.055>.
- [20] M.J. Saez-Lara, C. Robles-Sanchez, F.J. Ruiz-Ojeda, et al., Effects of Probiotics and Synbiotics on Obesity, Insulin Resistance Syndrome, Type 2 Diabetes and Non-Alcoholic Fatty Liver Disease: A Review of Human Clinical Trials, *Int J Mol Sci*. 17 (2016) <https://doi.org/10.3390/ijms17060928>.
- [21] R. Barnett, Obesity, *The Lancet*. 389 (2017) [https://doi.org/10.1016/s0140-6736\(17\)30273-8](https://doi.org/10.1016/s0140-6736(17)30273-8).
- [22] M. Sotak, M. Clark, B.E. Suur, et al., Inflammation and resolution in obesity, *Nat Rev Endocrinol*. 21 (2025) 45-61. <https://doi.org/10.1038/s41574-024-01047-y>.
- [23] Y. Rao, Z. Kuang, C. Li, et al., Gut *Akkermansia muciniphila* ameliorates metabolic dysfunction-associated fatty liver disease by regulating the metabolism of L-aspartate via gut-liver axis, *Gut Microbes*. 13 (2021) 1-19. <https://doi.org/10.1080/19490976.2021.1927633>.
- [24] Y. Han, Q. Ling, L. Wu, et al., *Akkermansia muciniphila* inhibits nonalcoholic steatohepatitis by orchestrating TLR2-activated $\gamma\delta$ T17 cell and macrophage polarization, *Gut Microbes*. 15 (2023) 2221485. <https://doi.org/10.1080/19490976.2023.2221485>.
- [25] S.R. Nagarajan, E. Cross, F. Sanna, et al., Dysregulation of hepatic metabolism with obesity: factors influencing glucose and lipid metabolism, *Proc Nutr Soc*. 81 (2022) 1-11. <https://doi.org/10.1017/S0029665121003761>.
- [26] G. Alyar, F.Z. Umudum, Differences in the levels of the appetite peptides ghrelin, peptide tyrosine tyrosine, and glucagon-like peptide-1 between obesity classes and lean controls, *Lab Med*. 55 (2024) 553-558. <https://doi.org/10.1093/labmed/lmae004>.
- [27] Y. Feng, W. Chen, J. Chen, et al., Dietary emulsifier carboxymethylcellulose-induced gut dysbiosis and SCFA reduction aggravate acute pancreatitis through classical monocyte activation, *Microbiome*. 13 (2025) 83. <https://doi.org/10.1186/s40168-025-02074-1>.
- [28] W. Wu, W. Kaicen, X. Bian, et al., *Akkermansia muciniphila* alleviates high-fat-diet-related metabolic-associated fatty liver disease by modulating gut microbiota and bile acids, *Microb Biotechnol*. 16 (2023) 1924-1939. <https://doi.org/10.1111/1751-7915.14293>.
- [29] Z. Luo, W. Xu, T. Yuan, et al., *Platycodon grandiflorus* root extract activates hepatic PI3K/PIP3/Akt insulin signaling by enriching gut *Akkermansia muciniphila* in high fat diet fed mice, *Phytomedicine*. 109 (2023) 154595. <https://doi.org/10.1016/j.phymed.2022.154595>.

- [30] Y. Luo, C. Lan, K. Xie, et al., Active or Autoclaved *Akkermansia muciniphila* Relieves TNF- α -Induced Inflammation in Intestinal Epithelial Cells Through Distinct Pathways, *Front Immunol.* 12 (2021) 788638. <https://doi.org/10.3389/fimmu.2021.788638>.
- [31] L. Lindberg, E. Hagman, P. Danielsson, et al., Anxiety and depression in children and adolescents with obesity: a nationwide study in Sweden, *BMC Med.* 18 (2020) 30. <https://doi.org/10.1186/s12916-020-1498-z>.
- [32] Y. Li, Y. Cheng, Y. Zhou, et al., High fat diet-induced obesity leads to depressive and anxiety-like behaviors in mice via AMPK/mTOR-mediated autophagy, *Exp Neurol.* 348 (2022) 113949. <https://doi.org/10.1016/j.expneurol.2021.113949>.
- [33] B. Liu, J. Liu, M. Wang, et al., From Serotonin to Neuroplasticity: Evolvement of Theories for Major Depressive Disorder, *Front Cell Neurosci.* 11 (2017) 305. <https://doi.org/10.3389/fncel.2017.00305>.
- [34] X. Xie, W. Li, Z. Xiong, et al., Metformin reprograms tryptophan metabolism via gut microbiome-derived bile acid metabolites to ameliorate depression-Like behaviors in mice, *Brain Behav Immun.* 123 (2025) 442-455. <https://doi.org/10.1016/j.bbi.2024.09.014>.
- [35] L. Khalili, G. Park, R. Nagpal, et al., The Impact of *Akkermansia muciniphila* on Mouse Models of Depression, Anxiety, and Stress: A Systematic Review and Meta-Analysis, *Curr Neuropharmacol.* (2025) <https://doi.org/10.2174/011570159X360149250225041829>.
- [36] H. Guo, X. Liu, T. Chen, et al., *Akkermansia muciniphila* Improves Depressive-Like Symptoms by Modulating the Level of 5-HT Neurotransmitters in the Gut and Brain of Mice, *Mol Neurobiol.* 61 (2024) 821-834. <https://doi.org/10.1007/s12035-023-03602-6>.
- [37] J. Liu, Y. Liu, C. Huang, et al., Quercetin-Driven *Akkermansia Muciniphila* Alleviates Obesity by Modulating Bile Acid Metabolism via an ILA/m(6)A/CYP8B1 Signaling, *Adv Sci (Weinh).* 12 (2025) e2412865. <https://doi.org/10.1002/advs.202412865>.
- [38] P. Gerard, Gut microbiota and obesity, *Cell Mol Life Sci.* 73 (2016) 147-162. <https://doi.org/10.1007/s00018-015-2061-5>.
- [39] I.M. Andrioaie, A. Duhaniuc, E.V. Nastase, et al., The Role of the Gut Microbiome in Psychiatric Disorders, *Microorganisms.* 10 (2022) <https://doi.org/10.3390/microorganisms10122436>.
- [40] J. Lu, H. Wu, S. Wu, et al., *Salmonella*: Infection mechanism and control strategies, *Microbiol Res.* 292 (2025) 128013. <https://doi.org/10.1016/j.micres.2024.128013>.
- [41] K.D. Tickell, R.L. Brander, H.E. Atlas, et al., Identification and management of *Shigella* infection in children with diarrhoea: a systematic review and meta-analysis, *Lancet Glob Health.* 5 (2017) e1235-e1248. [https://doi.org/10.1016/S2214-109X\(17\)30392-3](https://doi.org/10.1016/S2214-109X(17)30392-3).
- [42] A. Agusti, I. Campillo, T. Balzano, et al., *Bacteroides uniformis* CECT 7771 Modulates the Brain Reward Response to Reduce Binge Eating and Anxiety-Like Behavior in Rat, *Mol Neurobiol.* 58 (2021) 4959-4979. <https://doi.org/10.1007/s12035-021-02462-2>.
- [43] J.-J. Wen, M.-Z. Li, J.-L. Hu, et al., Different dietary fibers unequally remodel gut microbiota and charge up anti-obesity effects, *Food Hydrocolloids.* 140 (2023) <https://doi.org/10.1016/j.foodhyd.2023.108617>.
- [44] A.L. Hamilton, M.A. Kamm, P. De Cruz, et al., Luminal microbiota related to Crohn's disease recurrence after surgery, *Gut Microbes.* 11 (2020) 1713-1728. <https://doi.org/10.1080/19490976.2020.1778262>.
- [45] K. Wang, M. Liao, N. Zhou, et al., *Parabacteroides distasonis* Alleviates Obesity and Metabolic Dysfunctions via Production of Succinate and Secondary Bile Acids, *Cell Rep.* 26 (2019) 222-235 e225. <https://doi.org/10.1016/j.celrep.2018.12.028>.
- [46] T. Zheng, H. Hao, Q. Liu, et al., Effect of Extracellular Vesicles Derived from *Akkermansia muciniphila* on Intestinal Barrier in Colitis Mice, *Nutrients.* 15 (2023) <https://doi.org/10.3390/nu15224722>.
- [47] M.J. Liu, J.Y. Yang, Z.H. Yan, et al., Recent findings in *Akkermansia muciniphila*-regulated metabolism and its role in intestinal diseases, *Clin Nutr.* 41 (2022) 2333-2344. <https://doi.org/10.1016/j.clnu.2022.08.029>.
- [48] C. Zhou, S. Guo, P. Gong, et al., Nano-Selenium Alleviates Cd-Induced Chronic Colitis through Intestinal Flora, *Nutrients.* 16 (2024) <https://doi.org/10.3390/nu16091330>.
- [49] Q. Li, W. Liu, H. Zhang, et al., α -D-1,3-glucan from *Radix Puerariae thomsonii* improves NAFLD by regulating the intestinal flora and metabolites, *Carbohydr Polym.* 299 (2023) 120197. <https://doi.org/10.1016/j.carbpol.2022.120197>.

- [50] Y. Yuan, D. Wu, H. Chen, et al., Farnesol ameliorates DSS-induced IBD by regulating inflammatory cytokines, repairing the intestinal barrier, reversing the gut microbiota imbalance, and influencing fecal metabolome in C57BL/6 mice, *Biomed Pharmacother.* 180 (2024) 117518. <https://doi.org/10.1016/j.biopha.2024.117518>.